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THE INVERSE RELATIONSHIP BETWEEN DRUG AFFINITY AND EFFECTIVENESS: PREDICTION UNDER RATE THEORY, PARADOX UNDER OCCUPANCY THEORY

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ABSTRACT

The two major theories of drug action are occupancy theory (5) and rate theory (9). Although the two theories make a number of predictions that should serve to clearly distinguish one from the other, data generated over the last 25 years in intact muscle or organ systems have failed in this respect because the interpretation of those data has been confounded by unknown and unmeasurable factors such as the rates and extent of drug diffusion, uptake and metabolism. In the present communication rate theory and occupancy theory are discussed in the light of data obtained from a broken-cell system in which uncontrollable factors are minimized, the beta adrenergic-responsive adenylate cyclase of the S49 mouse lymphoma cell line. It is pointed out that rate theory predicts an inverse relationship between the affinity of isoproterenol (INE) for the beta adrenergic receptor and the magnitude of stimulation of adenylate cyclase. On the other hand, occupancy theory predicts that the affinity of INE and the magnitude of its effect will be directly related. By fitting data obtained in this system to the simple equations derived from the two theories it is demonstrated that the available data are in excellent agreement with the predictions of rate theory and diametrically opposed to the predictions of occupancy theory.

INTRODUCTION

In 1961 W.D.M. Paton (9) proposed that the magnitude of the effect produced by a drug-receptor interaction is a function of the rate at which the drug molecules associate with their receptors. Simply put, rate theory asserts that the activation of an effector is a quantum event -- a quantum of activation occuring each time a receptor becomes occupied. A corollary of

this rate theory is that agents which loiter on receptors as a result of slow rates of dissociation prohibit subsequent fruitful interactions; thus, the magnitude of stimulation caused by a drug would increase under conditions in which its affinity decreases.

At the time Paton offered his rate theory of drug action, occupancy theory, developed by A.J. Clark (2,3), had provided the theoretical groundwork of drug action for almost 40 years. Occupancy theory states that the magnitude of drug effect is related to the fraction of total receptors occupied by drug molecules. Although widely accepted by pharmacologists at the time (as well as at the present), Paton was dissatisfied with occupancy theory because of its failure to explain many experimental observations that form the very basis of pharmacology. For instance occupancy theory does not provide an explaination of why antagonists are antagonistic and why partial agonists lack full activity. (Rate theory explains these phenomena in terms of differing rates of dissociation between drug and receptor -- antagonists having slow rates of dissociation, agonists having rapid rates.) Ariens (1) and Stephenson (15) attempted to overcome this shortcoming of occupancy theory by proposing that the degree to which an agent activates an effector is a function of some quality of that agent -- its "intrinsic activity". Intrinsic activity is thus a theoretical, drug-dependent proportionality constant that relates fractional occupancy to magnitude of effect.

In recent years the rate theory-occupancy theory controversy has all but died away without having been settled by experimental evidence in favor of one theory or the other. Virtually all current theoretical work on drug action assumes, sometimes explicitly but more often implicitly , the validity of occupancy theory; models are therefore built upon that theory. Nowhere is this more true than in the study of drug activated adenylate cyclase systems. Models of drug stimulation of adenylate cyclase that are based upon the assumption that the enzyme is activated while the receptor is occupied include a variety of models from Gilman and co-workers (13,14,19), the 'terenary-complex ' model of DeLean et al (18) and models too numerous to mention devised by other groups active in this field . There are currently no models of drug activation of adenylate cyclase based upon rate theory. The widespread acceptance of occupancy theory by contemporary theoretical pharmacologists probably reflects the appreciation by those pharmacologists that the mathematical descriptions of drug action under occupancy theory are highly analogous to Michaelis-Menton analysis of enzyme activity, so familiar to those with a biochemical background. However, drugs are generally not substrates for the enzymes they activate and the fact that similar hyperbolic functions describe both drug activation of effectors and substrate activation of enzymes may be little more than a michevious coincidence of nature.

THE RELATIONSHIP BETWEEN AGONIST AFFINITY AND EFFECTIVENESS

Predictions based on rate and occupancy theories

It is the relationship between affinity and effectiveness that is, perhaps, the clearest and most experimentally accessible point of contention between rate theory and occupancy theory. As noted above, rate theory predicts that the degree of stimulation will increase as affinity decreases; occupancy theory predicts that as affinity increases so, too, will the degree of stimulation. Mathematical descriptions of these predictions are given in Equations 1 and 2 below, where the activity (A) of the system at equilibrium is given as a function of drug concentration ([D]), the rate constant of dissociation (k_2) and the dissociation constant (K_D).

Under rate theory:

$$A = \emptyset' \cdot S^{rt} \qquad \text{Eqn. 1}$$

where

$$S^{rt} = \frac{k_2[D]}{[D]+K_D} \qquad \text{Eqn. 1a}$$

Under occupancy theory:

$$A = \emptyset \cdot S^{ot} \qquad \text{Eqn. 2}$$

where

$$S^{ot} = \frac{[D]}{[D]+K_D} \qquad \text{Eqn. 2a}$$

It can be seen from these simple equations that the intensity of stimulus under rate theory (S^{rt}) is a function of the rate of association of the drug with its receptor, while the inetnsity of stimulation under occupancy theory (S^{ot}) is a function of the fractional occupancy of receptors. The terms $\emptyset$ and $\emptyset'$ relate the intensity of stimulus to the measured activity. These terms are not equal to one another, nor do they have the same units. Both terms incorporate drug-independent factors related to the ability of the effector to respond following drug-receptor interactions; however, the term $\emptyset$ incorporates an additional factor reflecting the "intrinisic activity" of the agent being tested. Since rate theory does not embrace the concept of "intrinsic activity", the term $\emptyset'$ remains constant for all agents whether they be full agonists, partial agonists or antagonists. (For a more rigorus treatment of these relationships see refs. 4, 6, 9, 10, 16 and 17.)

Empirical observations relating agonist affinity to effectiveness

The plasma membranes of the mouse lymphoma cell culture, S49, contain a drug responsive adenylate cyclase system that has been studied in great detail (see 19 and refs. therein). In this system, as well as many other adenylate cyclase systems, it is

possible to alter the affinity of an agonist for its receptor by manipulating the levels of GTP. For instance, in the presence of a minimal concentration of GTP (0.1 uM), the K_D of INE was found to be 80 nM , while in the presence of saturating concentrations of GTP (50-100 uM) the K_D of INE increased to 500 nM (13). When the ability of INE to stimulate adenylate cyclase under various concentrations of GTP was examined, it was found that there was 385% greater stimulation in the presence of 100 uM GTP than in the presence of 0.1 uM GTP. Thus, the stimulatory activity of INE increased as its affinity decreased. This inverse relationship between agonist affinity and effectiveness was first described in glucagon-sensitive adenylate cyclase preparations from liver membranes (11,12) and has since been observed in virtually every hormone-sensitive adenylate cyclase system in which it has been examined.

The relationship between the empirical observations and predictions on the theories

By inserting into Equations 1a and 2a the various K_D's reported for INE in the presence of high and low concentrations of GTP (13) one can calculate the alterations in stimulus intensity predicted by rate theory and occupancy theory as a function of GTP concentration. The predicted changes in these stimulus intensities can then be compared to the observed GTP-induced changes in stimulatory activity of INE. [In calculating S^{rt} it is necessary to have a value for k_2 . This is obtained from the K_D's provided by Ross et al. (13) by assuming that k_1 , the association rate constant, is invariant as GTP concentration is altered. That GTP affects primarily k_2 has been demonstrated (11,12) and is a generally held assumption in this field (8). For the purposes of the following calculations, k_1 is given the value of 10^8 min^{-1}.]

The results of the calculations described above are as follows: occupancy theory (Equation 2a) predicts that the stimulatory effect (S^{ot}) of 1 uM INE will decrease from 0.93, in the presence of 0.1 uM GTP, to 0.67 in the presence of 100 uM GTP -- a decrease of 28%. Rate theory, on the other hand, predicts that the stimulatory effect (S^{rt}) of 1 uM INE will increase from 7.41 to 33.3 as the GTP concentration is raised from 0.1 uM to 100 uM. This represents a 349% enhancement in the stimulatory activity of INE, a figure to be compared to the 385% increased stimulation actually observed (see above). Thus, the prediction on rate theory is in excellent agreement with the observed GTP-induced enhancement of stimulation by INE, while the prediction on occupancy theory fails to coincide with the experimental observations even in a qualitative manner.

There are other broken-cell systems in which it is clear that drug affinity is inversly related to effectiveness, as predicted by rate theory. For instance, since the development of

radioligand binding assays it has become a commonplace finding that antagonists have higher affinities for receptors than agonists do -- essentially a re-statement of rate theory in empirical terms. That antagonists display slow rates of dissociation was inferred by Paton and others who observed slowly reversible antagonism when competitive antagonists were washed out of intact muscle systems. This was one observation, inexplicable under occupancy theory, that led Paton to propose his alternative theory.

Thermal perturbations of receptor/effector systems have also revealed an inverse relationship between affinity and effectiveness. Insel and Sanda (7) observed in the S49 lymphoma cell line that the stimulatory effect of INE on adenylate cyclase increases as temperature increases even though the affinity of INE declines.

CONCLUSIONS

The above arguments represent an attempt to highlight a conflict between rate theory and occupancy theory that may now be amenable to analysis as a result of recent advances in research technology. During the 1960's and early 1970's the virtues of rate theory and occupancy theory were actively debated. Unfortunately, the experimental work designed to prove or disprove one theory or the other was carried out in smooth muscle or similar intact tissues where unknown and uncontrollable experimental factors made unequivocal interpretation of the data impossible. Today pharmacologists have access to systems and techniques that obviate such problems. Adenylate cyclase systems, in which entire receptor/effector systems are contained in the volume of a few microliters, eliminate uncertainties regarding drug diffusion, uptake or metabolism. Radioligand binding techniques offer the means of quantifying binding parameters without relying on approximations of experimentally inaccessible rate constants inferred from the activation of effector components in intact tissues. Electrophysiology, particularly patch-clamping, affords the opportunity to examine the effects of drugs at very brief intervals following application, thereby providing access to short-lived phenomena predicted by rate theory; e.g., "fade" and short-lived stimulation by antagonists (9,10).

Upon rigorous examination of a variety of receptor regulated enzyme or ion-gate systems the inverse relationship between affinity and effect may be found to be so invariant as to be considered a general law of drug action. However, it would appear that such a relationship cannot co-exist with occupancy theory unless one expands the concept of "intrinsic activity" to the point that it totally distorts the original concept that fractional occupancy is directly related to effect -- at which point occupancy theory is no longer occupancy theory.

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